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A diazotized mixture of luminol and 3-amino-L-tyrosine prepared as an acetone-precipitated solid polymerizes slowly (over a period of weeks) yielding a brown, water soluble polymer which migrates electrophoretically as an anion at pH 6. Solutions of this substance in D_2O or a mixed D_2O/deuterated DMSO solvent present very broadened ^1H-nmr resonances, and all attempts to chemically fragment this material into its subunit structures have been unsuccessful. The polymer, which has been named diazoluminomelanin (DALM) because of a possible relationship to the natural melanins, is of interest because its aqueous solution with alkaline H_2O_2 and bicarbonate ion flashes a transient chemiluminescence on being irradiated with an intense pulse of microwave energy. Also, aqueous solutions of DALM serve as substrates for the green heme proteins.

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NMR Characterization of Products Formed in Diazotizing Mixtures of
Luminol and 3-Amino-L-tyrosine   (UNCLASSIFIED)

19.   The reactions leading to DALM appear to involve chiefly 3-diazonium-L-
tyrosine and the 5-diazonium derivative of luminol combined in an
intimate solid matrix. This solid presents a strong esr absorption,
and the polymerization may involve both homolytic and heterolytic
processes. Characterization of a polymer obtained from diazotized
aqueous solutions of 3-amino-L-tyrosine alone indicate a linkage based
on displacement of the 3-diazonium group by the alpha-amino nitrogen
atom, i.e., an aryl amine polymer which retains the structural
qualities of 3-amino-L-tyrosine. The DALM polymer might contain
similar structural features, but this has not been established.

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NMR Characterization of Products Formed in Diazotizing

Mixtures of Luminol and 3-Amino-L-tyrosine

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FINAL REPORT

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1. INTRODUCTION

An interest in this project came about as a result of the principal investigator's involvement in a summer faculty research program at Brooks AFB, May - August, 1988. The chemical system under investigation here originates from a reaction involving luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), 3-amino-L-tyrosine (hereafter, 3-AT) and sodium nitrite in a mixed dimethylsulfoxide/water solvent (diazotization conditions). This mixture produces a brown, anionic, polymeric product which chemiluminesces modestly but over long time intervals in basic aqueous solutions containing hydrogen peroxide, and the chemiluminescent reaction is enhanced in the presence of carbon dioxide (1). The name diazoluminomelanin (DALM) is in recognition of the substance's origin from a substituted tyrosine (biological melanins derive from L-tyrosine), and from its color (2). The significance of DALM comes from the finding that aqueous solutions of DALM containing alkaline hydrogen peroxide flash in chemiluminescence when subjected to intense pulses of microwave energy (3). These effects occur even when single pulse fluences do not significantly heat the target sample. This is an important phenomenon since it shows that under certain conditions (e.g., at high incident power levels), the absorption of microwave energy can bring about the bond-breaking processes of regular chemical reactions. In this case the photonic energy ratio (visible light/microwave) is on the order of 10^5, and it does not seem reasonable that molecular absorptions at rotational energy levels could cooperate to achieve electronic transition energies (e.g., by

an anti-Stokes process). Instead, the microwave-induced chemical reactions may be a result of the acoustic waves (4) which are generated when a target absorbs high powered microwave pulses. Such shock waves can cause large instantaneous temperature excursions, opening the possibility of free radical production and thus a mechanism for initiating luminol's chemiluminescence (5).

The chemistry of DALM itself has been a source of confusion and irreproducibility, and the resolution of these problems along with structural characterizations of DALM, to the extent possible, is the main purpose of the work reported here. As used in the earlier studies at Brooks AFB, solutions of DALM contained the anionic substance described above, unreacted sodium nitrite, unreacted luminol and other components. Is DALM just the brown anionic polymer, or is it responsible for producing free radicals, which in turn activate the free luminol in the mixture? Nitrite has been observed to enhance chemiluminescence reactions, as in the case of CO_2 (1), so it may have been involved, too. Another problem originated from confusion about the conditions needed to obtain the brown anion. It turned out that this reaction did not take place in the diazotizing solution but instead resulted from "curing" the acetone-precipitated solid at length, a reaction which appears to involve the polymerizing reactions of diazonium groups. Some of these problems were resolved in the work reported here.

2. OBJECTIVES

The main objective of this project is a structural characterization of a polymer prepared by diazotizing a mixture of

luminol and 3-amino-L-tyrosine. The work also seeks to characterize the intermediate compounds, radicals, etc., leading to this polymer, and comparisons with similar substances such as a polymer obtained by diazotizing 3-amino-L-tyrosine alone is expected to be pertinent to the main objective. The project especially seeks to understand why aqueous solutions of the polymer of luminol and 3-amino-L-tyrosine emit a transient luminescence on absorbing an energetic pulse of microwave energy. These objectives do not alter the intent of the original research proposal.

3. EXPERIMENTAL METHODS

3.1. Materials.

Luminol, 3-amino-L-tyrosine (3-AT) and sodium nitrite were purchased from Sigma Chemical Co., and the various deuterated solvents such as DMSO, deuterium oxide, etc. were obtained from Aldrich. These materials were used without further purification.

3.2. Reaction procedures.

3.2.1. Diazotization polymer of 3-amino-L-tyrosine (alone).

A quantity of 296 mg of 3-AT as the dihydrated hydrochloride is dissolved in 25 mL deionized water to obtain solution A. Solution B is prepared by dissolving 69 mg $NaNO_2$ in 25 mL deionized water. Solutions A and B are then thoroughly mixed and the container is sealed with parafilm. The solution turns brown in a matter of minutes, and it is stored at room temperature for nine days. After this period the polymer is precipitated with 400 – 500 mL of acetone. After discarding the supernatant, the solid is

again washed with 50 mL pure acetone. This reaction does not require a mixed solvent (e.g., DMSO/water) since 3-AT is water soluble.

3.2.2. The preparation of DALM.

The diazotization reaction leading to DALM requires a mixed solvent system since luminol is virtually insoluble in pure water. The method for preparing DALM is as follows: 27.5 mg of 3-AT are dissolved in 10 mL water, and 11.2 mg $NaNO_2$ is then quickly dissolved in this solution. This mixture is allowed to stand for 5 minutes, during which time it turns brown. After the delay 21.9 mg luminol dissolved in 10 mL DMSO is combined with the brown mixture and allowed to react for a further 30 minutes; then the products are precipitated with 200 mL acetone and the dried solid is allowed to age at room temperature for about a month. DALM is formed during the latter aging reaction.

3.3. NMR Spectra.

Nmr spectra were obtained with a Chemagnetics A-200-FT spectrometer, which has multinuclear capabilities. This instrument now has programmable bi-level decoupling and is able to operate in various 2D and even 3D data acquisition modes. The instrument was recently interfaced to a stand-alone 80386/80387 computer with a 124 MB hard disk, and the computer uses FELIX software for multidimensional data analysis. This much improved system was provided through NIH funds, and it frees the nmr facility of the problems which will arise when tne university adopts a new central

computer system.

3.4. Differential scanning calorimetry.

Differential scanning calorimetry was was carried out on milligram quantities of samples using a Perkin-Elmer Model DCS-4 instrument operating under the TADSOFT system.

3.5. Other physical methods.

Measurements of chemiluminescence were obtained by means of a Turner model TD-20e luminometer. This instrument has a light guide for communicating the photometer with 2.4 GHz microwave and 20 kHz acoustic irradiation cells. Infrared spectra were obtained with a Bomem FTIR instrument. UV/visible spectra and pH readings were obtained using various bench instruments.

3.6. Electrophoresis.

Electrophoretic separations were carried out on 2.54 cm x 15 cm Sepraphore III strips soaked in a pH 6 acetate buffer. The applied DC field was 10 V/cm. Densitometry was accomplished with a Gelman ACD-18 zone scanner.

3.7. Molecular orbital computations.

An IBM PS/2 was used to perform Huckel molecular orbital computations using Trinity Software's HMO program, which is graphics oriented and may use either zero order parameters or the Streitweiser parameters.

4. RESULTS AND DISCUSSION

4.1. Diazotization of luminol and 3-amino-L-tyrosine: the immediate products.

The reactions of both luminol and 3-AT with the diazotizing reagent ($NaNO_2$), either separately or mixed together in a DMSO/water solvent (or in the case of 3-AT, pure water), should yield diazonium salts as the immediate products since luminol and 3-AT are aromatic amines (6). Fig. 1 presents [1]H-nmr spectra of the beginning reactants (luminol and 3-AT) and of the products obtained after adding $NaNO_2$ to these. The results are consistent with straightforward diazotizations. The insets at the right-hand side of Fig. 1 show graphically the net pi-electron charges at numbered atom positions (the meaning of the numbering is shown in the respective structures), and the values were obtained by Huckel molecular orbital (HMO) computations using zero-order parameters. These net charges are superimposed on the ring current effect and should correlate with aromatic chemical shifts; however, one should expect only general trends rather than quantitative predictions. The scale center position is neutrality. Values to the left are electron deficient (deshielding) while values to the right are electron excesses (shielding).

The lower spectrum in Fig. 1 shows the aromatic resonances of 3-AT, which occupy a range of only about 0.5 ppm and are thus in good agreement with the HMO computation. The second spectrum (second from bottom) was obtained from the immediate diazotization product of 3-AT. The HMO computation predicts electron deficiencies at carbons 2 and 6 and a downfield shifting of their

Figure 1 – Proton nmr spectra and HMO computations for luminol, 5-AT and the respective diazonium derivatives
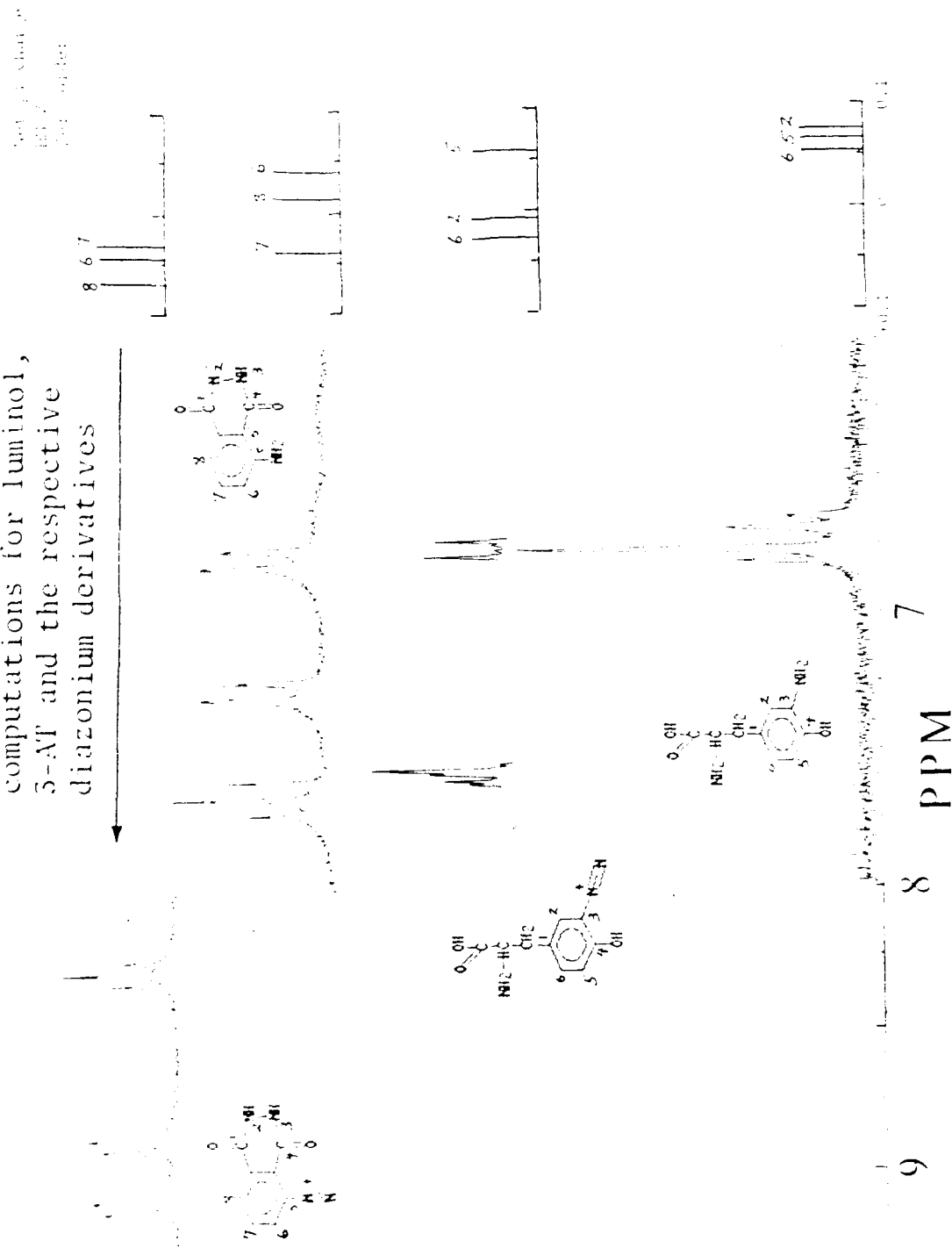
respective hydrogen substituents, as observed in the spectrum. It should be noted that the aliphatic alpha and beta hydrogen resonances were only minimally shifted after diazotization.

The third spectrum was obtained from luminol in deuterated alkaline aqueous solution (the neutral species is not water soluble), and the HMO calculation for the anion is in good agreement with the observed spectrum, an exception being the positioning of the middle doublet (presumed H-8). The hydrogen at position 7 should be the most downfield shifted resonance and apparently is since it shows the expected triplet splitting due to two hydrogens in proximity. The top nmr recording was obtained from luminol diazonium cation in heavy water. The HMO computation predicts an upfield triplet (H-7, as observed) and a substantial downfield shifting of hydrogens 6 and 8; however, much larger downfield shifts are seen for the latter two in the observed nmr spectrum, and H-7 is also somewhat downfield from its position in luminol. In pure deuterated DMSO the neutral (diazo) form of this compound (spectrum not shown) is the dominant species. The broad azine hydrogen resonance moves upfield and is superimposed on resonances due to the aromatic hydrogens. The latter have shifts intermediate between the cationic and anionic species shown in Fig. 1; however, while the HMO computation predicts an intermediate shift, the order of the doublets and triplet is wrong.

Mixtures of luminol and 3-AT react quantitatively with $NaNO_2$, yielding the two diazonium compounds described above. Using ^1H-nmr it was possible follow the further reactions of these substances in a deuterated $DMSO/D_2O$ solvent; however, as will be shown in 4.3.

(below), the substance of interest (DALM) forms in an acetone precipitate of the immediate products, i.e., in a solid containing a mixture of the 3-AT and luminol diazoniums. These compounds do react slowly in the mixed solvent, and the nmr resonances of 3-diazonium-L-tyrosine vanish fairly rapidly (in a few days) and are replaced by broadened resonances. The diazonium product of luminol is more persistent at room temperature.

4.2. Diazotization polymerization of 3-amino-L-tyrosine (alone).

A polymer is obtained from diazotized solutions of 3-AT, and Maj. Eric Holwitt (Brooks AFB) used the acronym DAT (for diazotized tyrosine) to describe this substance. Maj. Holwitt's method for preparing the polymer is as follows: the diazotized product is allowed to stand for 9 days in the pure water solvent and is then precipitated by acetone. After further washings with acetone DAT is obtained as a brown, water soluble solid.

A neutral solution of DAT is brown, but the solution changes to red in the fashion of an indicator when it is titrated with sodium hydroxide. The DAT polymer does not show evidence of extensive double bond conjugation since the solid is brown and its solutions are not extremely dark. In contrast, the pure solids of extensively conjugated substances (such as a melanins, chlorophylls, etc.) should be nearly black to the eye and concentrated solutions should also be nearly black. Electrophoresis on Sepraphore III strips in an acetate buffer at pH 6 reveals that DAT is an anion.

The bottom [1]H-nmr recording of Fig. 2 was obtained from DAT in
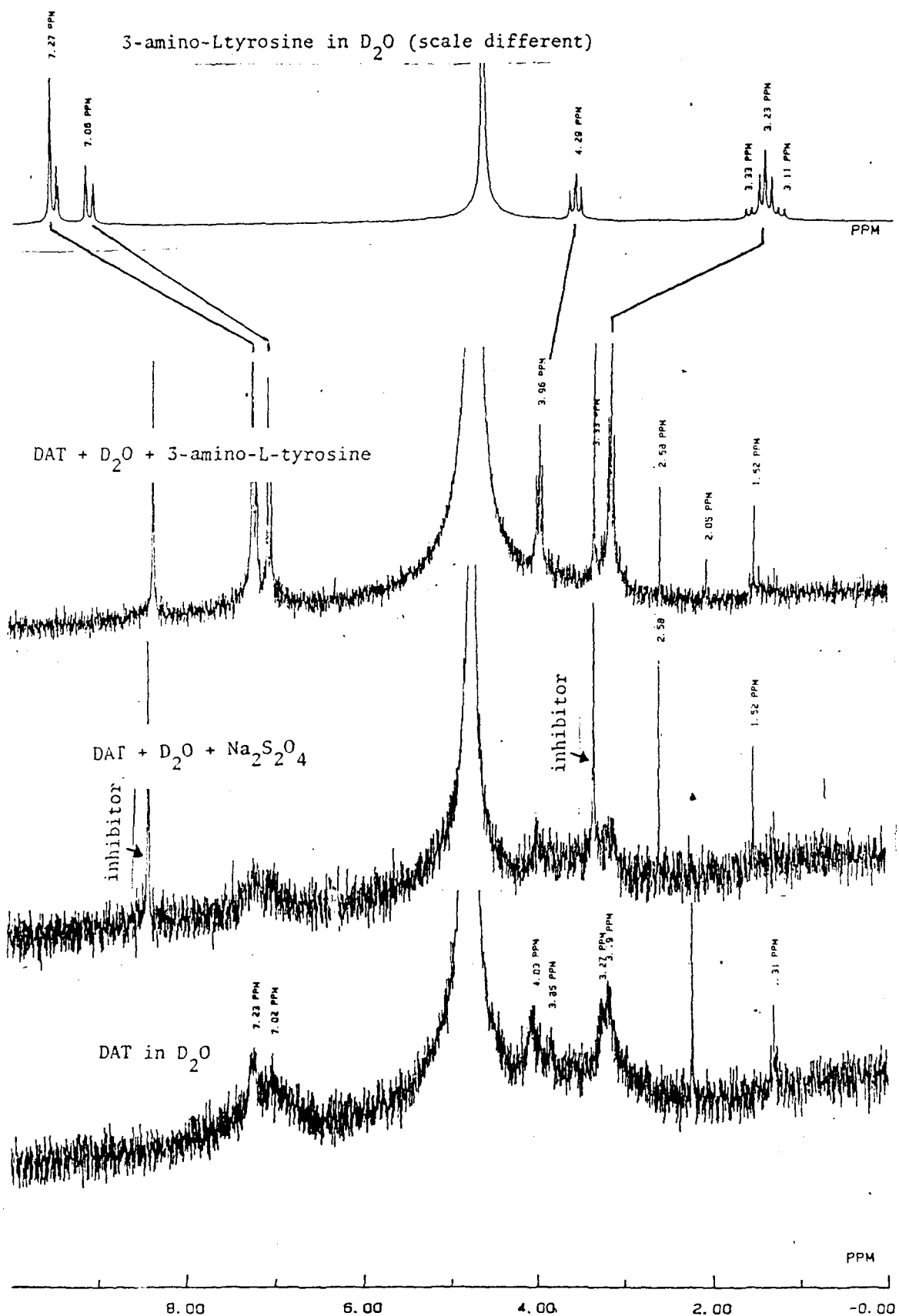
Wright 12

Figure 2 - Proton nmr of the 3-AT polymer

heavy water and it presents the typically broadened resonances of a polymer. The second spectrum (from the bottom) was obtained after sodium hydrosulfite ($Na_2S_2O_4$) was added to the DAT solution. The hydrosulfite ion reductively cleaves $-N=N-$ groups (7). Retention of the broad lines in the second spectrum argues against much involvement of $-N=N-$ linkages in the polymer backbone; however, this reagent did partially decolorize the solution, indicating a break-up of some kind of conjugation. Note that the two narrow lines in the second spectrum are due to contaminants which originated in the commercial hydrosulfite preparation.

The third spectrum of Fig 2 shows the effect of adding a small amount of 3-AT to the DAT solution. A spectrum of pure 3-AT in heavy water is shown at the top for reference (note that Figs. 1 and 2 are not exactly comparable due to solvent effects). The result obtained in the third spectrum shows clearly that the line broadening observed for the polymer is not due to field homogeneity problems (as might be caused by sediments) since a compromise of homogeneity would have also broadened the resonances of 3-AT. More significantly, it is seen that the narrow lines of 3-AT are almost exactly coincident with the broadened lines of the polymer. Recalling that the spectrum of diazotized 3-AT is significantly altered from that of 3-AT (see 3.1.), its further reaction clearly involves the 3-diazonium group. The data thus suggest that the DAT polymer must retain much of the nature of 3-AT itself, and this finding substantially restricts the kinds of structures which we may write for the polymer. Some of the possibilities which might occur in a polymerization of 3-diazonium-L-tyrosine are shown in

Fig. 3.

The structure proposed for DAT in Fig. 3A is the one most consistent with all of the above cited evidence for the following reasons: 1.) considering the reaction conditions, it is not completely unreasonable to expect that the alpha-amino group of 3-diazonium-L-tyrosine would enter an aromatic displacement reaction (8) at the 3-position of the benzenoid ring, though slowly since the conjugate base of the amine is a requisite for such a displacement. The involvement of an amine at pH 6 is not typical and dihydroxyphenylalanine (DOPA Fig. 3B) should be formed in a competing hydrolysis reaction; 2.) the resulting polymer structure (Fig. 3A) is not expected to show large chemical shift differences since the motif is very similar to 3-AT; 3.) because the alpha amino group becomes aromatic, the zwitterionic quality of 3-AT would be lost, and the carboxylate groups would impart the observed anionic property; and 4.) the phenolic -OH group should titrate near pH 10 (9) and might be the reason for the indicator qualities of the substance (granting the existence of some degree of extended conjugation in the polymer). Since the alpha hydrogen is near an aromatic ring there is some worry that it might experience a ring current shift; however, the aryl-N-CH bond angles might average this hydrogen near a ring current crossover zone.

Diazonium displacements involving the other functional groups would not lead to the observed properties of DAT (see the Appendix A tabulation). For example, if the carboxylate group were involved, the resulting polymer should be cationic (Fig. 3C), and a phenolic displacement leads to a zwitterion with a net neutral

Figure 3 - Possible polymer structures
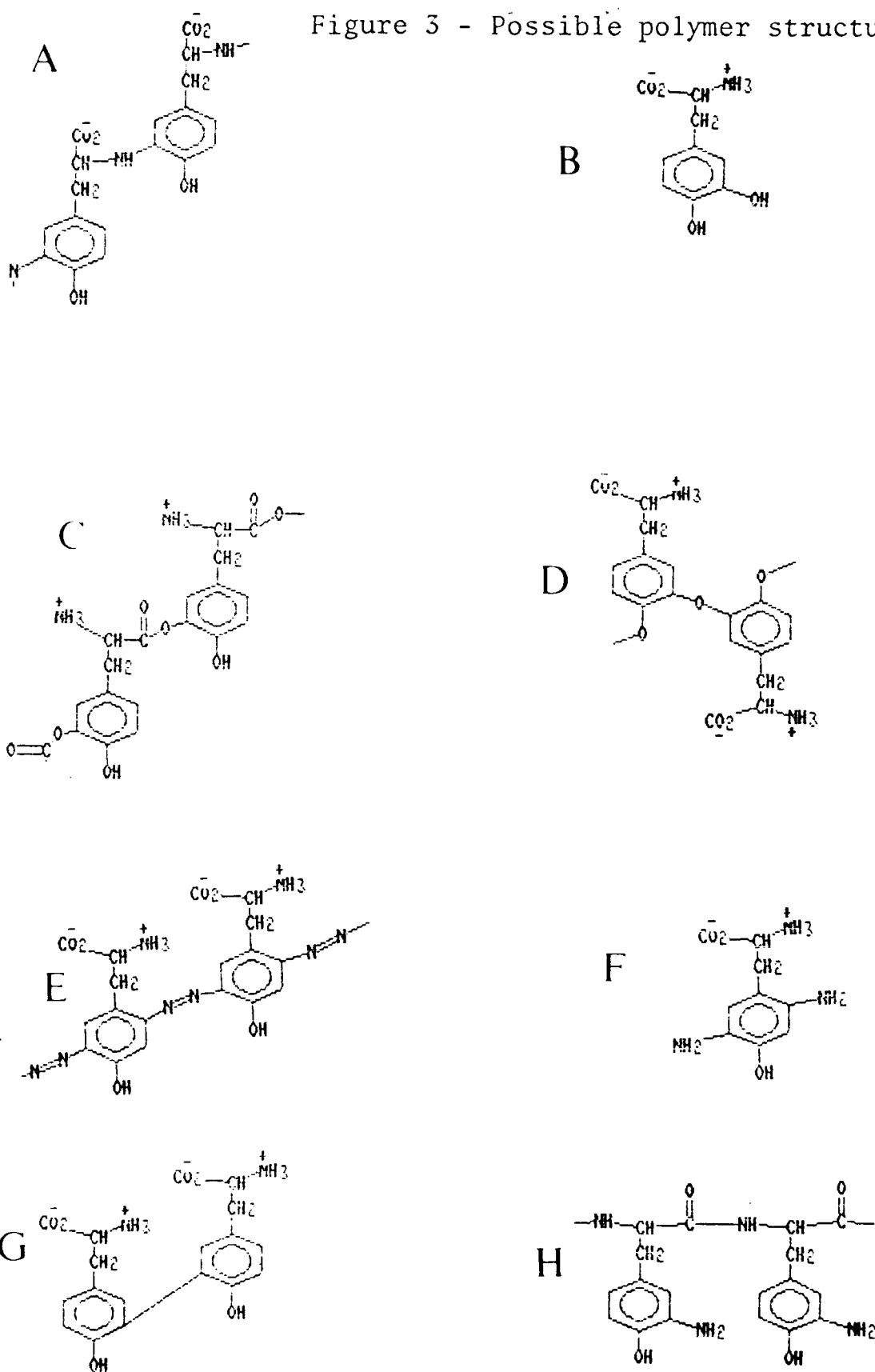
charge (Fig. 3D) with no reason for the observed indicator properties. The diazo coupling (Fig. 3E) and its isomeric variants are reasonable possibilities and these involve extensive structural conjugation, which is inconsistent with the relatively modest pigmentation observed for this polymer. Also, a diazo-coupled polymer should have been cleaved by $Na_2S_2O_4$, leading to aromatic diamines of the type shown in Fig. 3F. Biaryl coupling products (Fig. 3G) are favored in an alkaline solvent (10), and the actual reaction conditions were near neutral. A biaryl coupling should lead to dimers instead of extended polymers. Finally, the peptide (Fig. 3H) would be uncharged at neutral pH, and the reaction conditions and clear evidence of ring functionalization at the 3-position simply do not indicate this kind of product.

Fig. 4 suggests how a partial oxidative process might extend the conjugation in some of the residues, and HMO calculations (see Appendix B) show that two double bonds in conjugation with the aromatic ring would shift the absorption into the visible spectrum; furthermore, titration of the phenolic -OH to the anionic phenolate form could shift the absorption band by as much as 20 percent in the case of the structure of Fig. 4A. These are speculations, and other conjugative modes may be acting as the cause of the observed pigmentation.

It cannot be said that a similar polymeric backbone is involved in DALM since the reaction leading to DALM occurs in a solid matrix rather than in solution. Analogous polymerizations of 3-AT have not been attempted at this time.

A

B
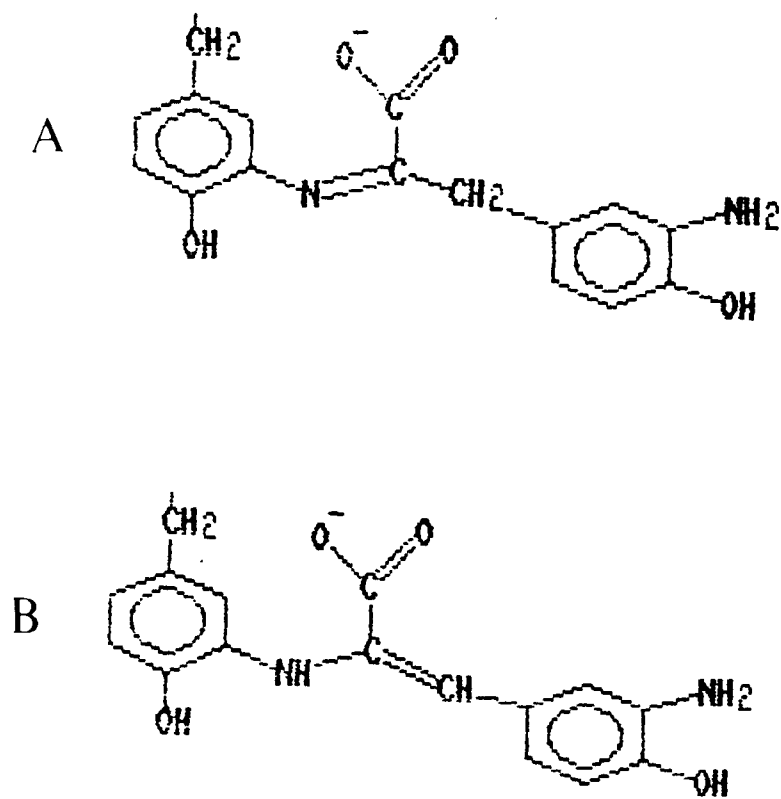
As shown here, the introduction of just one double bond by dehydrogenation brings the ring and carboxylate groups into conjugation, shifting absorption into the visible spectrum. Structure B is more delocalized and titration of the phenolic hydrogen causes a red-shift of the absorption bands (see Appendix B).

Figure 4 - Possible extensions of conjugation

4.3. Diazotization polymerizations of mixtures of luminol and 3-amino-L-tyrosine (DALM).

A chemiluminescent, water-soluble, anionic polymer described as diazoluminomelanin (DALM; see reference 2) has been obtained from the diazotized products of a mixture of luminol and 3-AT. Fig. 5 shows the electrophoretic properties of this substance at pH 6 with regard to its color, UV fluorescence and chemiluminescence (the latter being activated by alkaline H_2O_2). It should be noted that under these conditions the DAT polymer (4.2.) migrates at about half the rate of DALM in a considerably elongated band. Crude DALM preparations (see 3.2.2.) contain a substantial amount of unreacted $NaNO_2$ and are brown; however, in the absence of admixed $NaNO_2$ the solid is very dark, indicating an extensively conjugated structure.

4.3.1. Effect of varying nitrite concentration.

There is not a good large scale preparative method for purifying DALM, and the crude product is contaminated with unreacted $NaNO_2$. It can be desalted on a Sephadex G-50 column using pure water as the eluent; however, DALM and especially the diazonium derivatives of luminol and 3-AT apparently react with Sephadex. The pigments adhere permanently, and columns have to be discarded after one separation. Diazotized luminol elutes more slowly than Sephadex salt fractions. The diazotizing reagent ($NaNO_2$) presumably reacts in a 1:1 ratio with the total aryl amine content of the starting reactants, and one thought has been to decrease the amount of nitrite in the starting mixture so that it
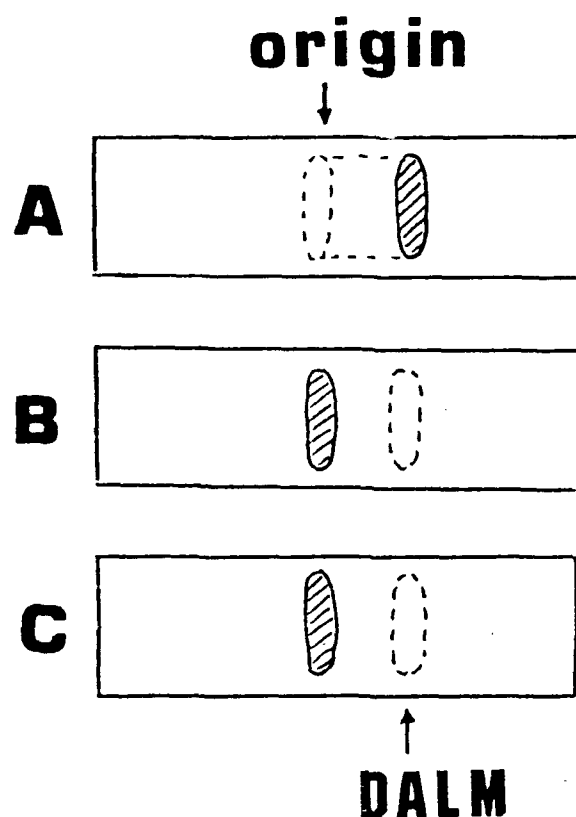
Electrophoresis of DALM on Sepraphore III strips. The buffer is 0.1 M acetate, pH 6. Separation was carried out at 15 V/cm for 30 minutes. Anions migrate to the right. A: Color - DALM carries most of the color. B: Alkaline H_2O_2 blot. Chemiluminescence of the DALM band (weak) was enhanced under CO_2. C. Long wave UV fluorescence. DALM and the origin band fluoresce blue.

Figure 5 - Electrophoresis of DALM

is effectively used up, or at least nearly used up. Fig. 6 shows this type of variation. The data of Fig. 6 were obtained from solutions which were .0033 M each in luminol and 3-AT, and the nitrite:total aryl amine ratios for this family of curves is as follows: 5:1 (crosses); 2.5:1 (circles); and 1:1 (squares). All three curves approach the same plateau, and it is seen that the diazotization is best done with a stoichiometric amount of nitrite and a longer reaction time. A slight excess of nitrite is probably advisable since the diazonium formation reaction is known to be second order with respect to nitrite (11).

4.3.2. Evidence indicating that DALM forms in the acetone precipitate.

In our continuing studies of DALM it was discovered that fresh acetone precipitates of the diazotization product mixture do not contain this anionic pigment. The substance which we are calling DALM originates in a slow reaction when the acetone precipitated material is allowed to stand at length, literally for weeks. Insight into the nature of this process came from studies of the diazotization of luminol alone, which produces the luminol diazonium cation (12). The latter is stable enough to be isolated as the acetate or chloride salt without an explosion. Differential scanning calorimetry (DSC) reveals that this substance decomposes exothermically near 150 C, as shown in Fig. 7A. This exotherm is consistent with a decomposition of the diazonium functional group. A similar exotherm occurs in the immediate acetone precipitate of diazotized mixtures of luminol and 3-AT but at a temperature closer

VARIATION OF NITRITE IN DALM
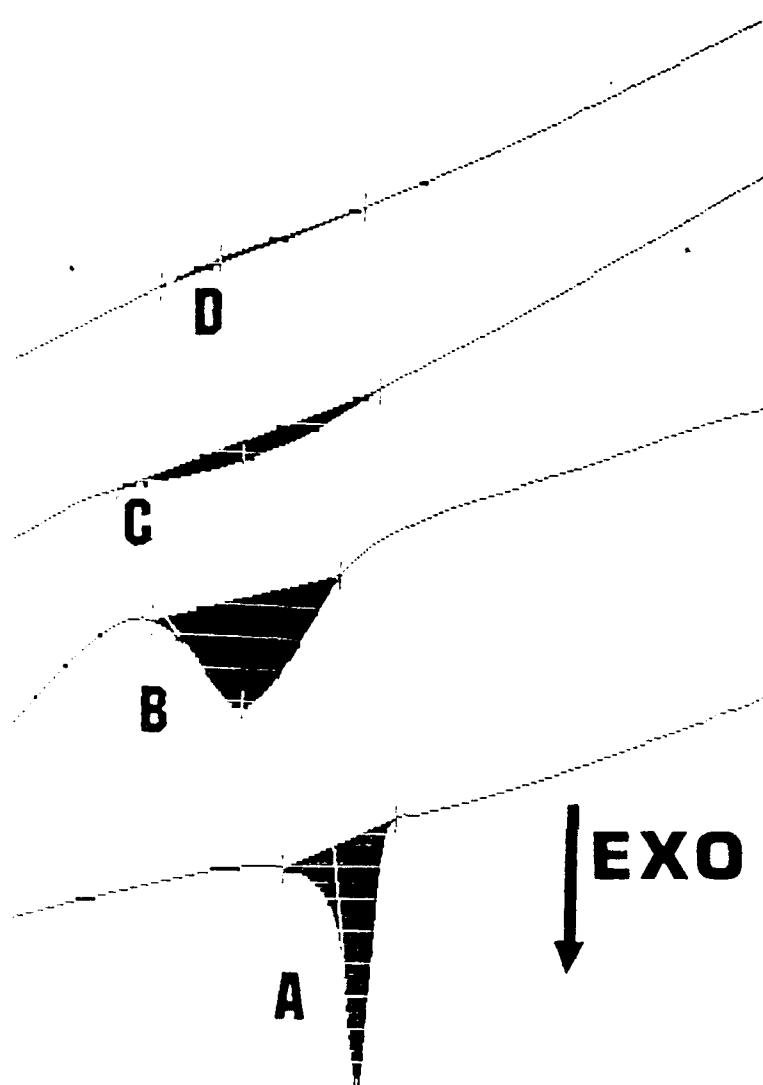
Figure 6

ABSORBANCE AT 400NM

TIME IN SECONDS
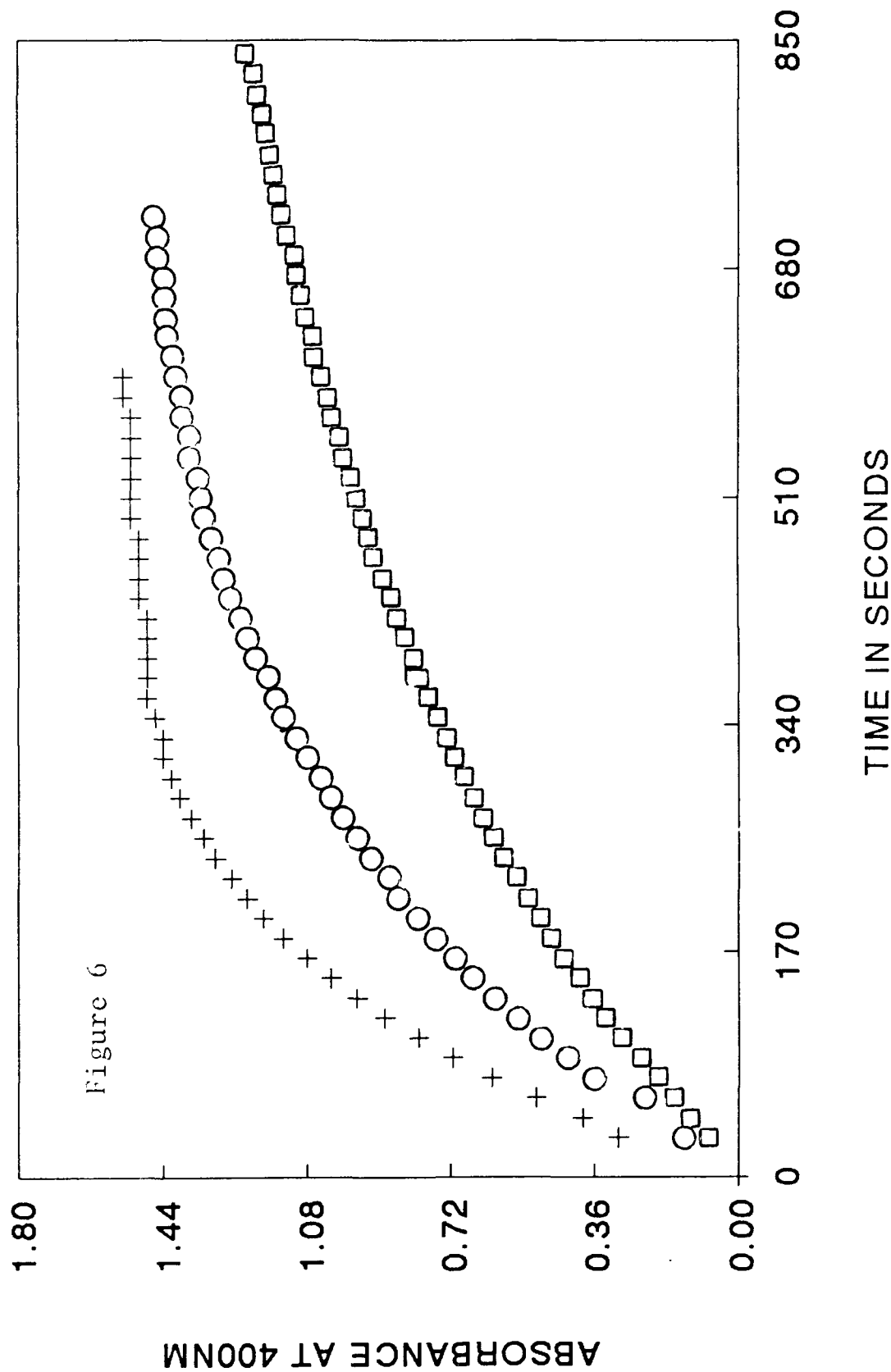
Figure 7 — Differential scanning calorimetry recordings obtained
with a Perkin-Elmer Model DCS-4 operating under TADSOFT. Exotherms
are downwards. A: Luminol diazonium salt (enriched, peak exotherm
151 C, -92.5 cal/g). The following data were obtained from DALM-
forming acetone precipitates. B: Fresh acetone precipitate (peak
exotherm 123 C, -30.8 cal/g). C: Acetone precipitate aged 12 days
at 25 C (peak exotherm 119 C, -8.5 cal/g). D: Acetone precipitate
aged 24 days at 25 C, virtual blank. The exotherm values (peak
temperature and specific heat) are based on instrument
computations, and recordings A - D do not have the same vertical
scale. All of these preparations contained unreacted sodium
nitrite and the exotherms do not represent pure compounds.

to 120 C. As shown in Fig. 7B-D, this exotherm fades away on a time scale comparable to that of the formation of DALM, indicating the involvement of the diazonium groups in the polymerization. Since the structure of DALM remains obscure, the nature of the reaction is also unclear. It might involve a displacement of molecular N_2 in an aromatic S_N1 process (8) similar to that considered for the DAT polymer (see 4.2.); however, this solid presents a strong esr absorption (2), and it is clear that homolytic reactions are taking place. Diazonium compounds react heterolytically in neutral or slightly acidic aqueous solutions, but homolytic decompositions leading to free radicals becomes the dominant mode in organic solvents and in alkaline aqueous solutions (10). Competing homolytic and heterolytic processes might occur in the DALM-forming solid.

The temperature dependence of the DALM-forming reaction was explored during the reporting period. When the diazotized solid was stored at 4 C the reaction processes were suspended for many weeks. The reaction was predictably faster in a 37 C thermostated compartment, but the product contained mostly insoluble material. Room temperature (ca. 25 C) appears to be the best condition for this reaction.

4.3.3. ^{1}H-Nmr spectra of DALM.

Proton nmr spectra of the immediate DALM-forming reaction mixture (Fig. 8A) clearly show the high resolution absorption lines of the diazonium derivatives of luminol and 3-AT, but on standing at length as the acetone precipitate these give way to the
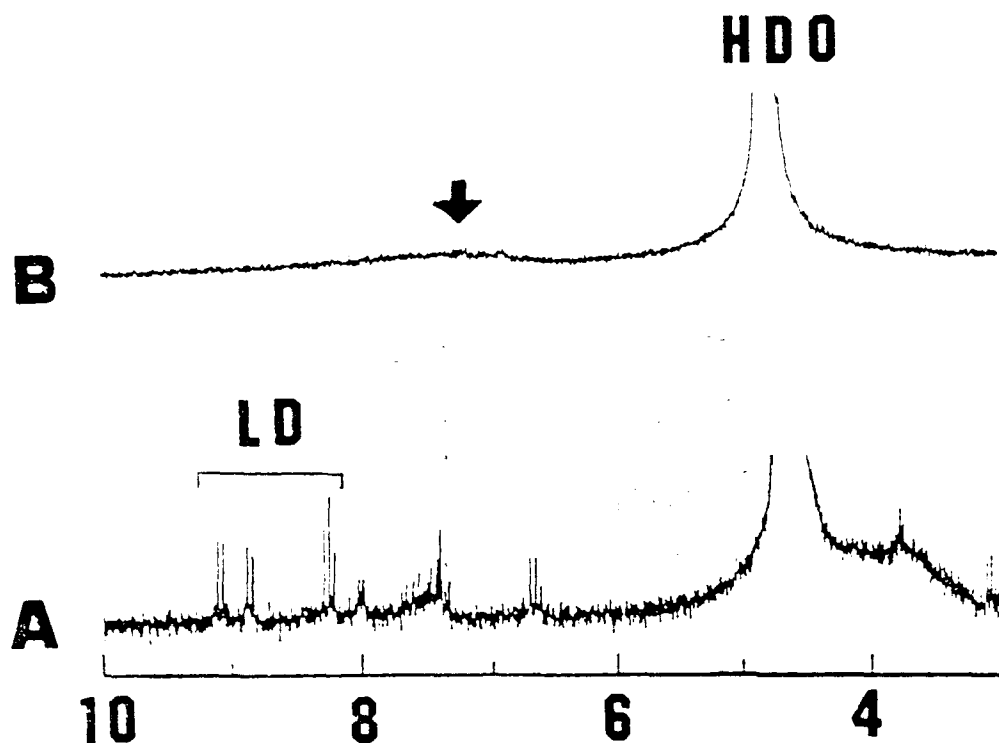
Figure 8 - ^{1}H-nmr spectra obtained at 200 MHz using a Chemagnetics A-200 spectrometer. A: The immediate reaction product in deuterated DMSO/D$_2$O, 16 acquisitions. The doublet-doublet-triplet of luminol diazonium (LD) is marked. B: The aged (1 month) DALM-forming solid in D$_2$O, 100 acquisitions. Only a broad resonance (arrow) is seen (further experiments in which low molecular weight substances were added to DALM solutions produced narrow lines; thus, the observed broadening is not due to sediments). The Scale is in PPM.

broadened, featureless resonances typical of polymeric substances (Fig. 8B). All attempts to chemically fragment or depolymerize DALM into its structural subunits have met with frustration (see 4.4., below). We are continuing in these efforts, and if fragmentations are achieved it should be possible to apply the more definitive structural methods, including 2D nmr.

4.4. Attempts to cleave the polymer into smaller subunits with oxidizing or reducing agents.

The DALM polymer is stable, but some substances are reactive with it, altering its nmr spectrum or other properties. Only a partial bleaching of the pigment was obtained with $KMnO_4/Na_2SO_3$, and the purple of MnO_4^- faded slowly. It was necessary to separate DALM and permanganate by means of electrophoresis and apply densitometry to observe this effect. The reagent $Na_2S_2O_4$, which reductively cleaves diazo (-N=N-) linkages (7) produced the nmr alterations shown in Fig. 9 (i.e., introduction of a new but still broadened absorption near 4 ppm) and achieved a partial bleaching of the pigment. NaClO produced a similar effect (Fig. 10); this reagent changed the pigment to yellow and then to colorless. The reducing reagent $NaBH_4$ caused a slow but complete bleaching of the pigment. The borohydride reagent caused the broad DALM resonances above and below the HDO resonance to vanish (Fig. 11), and the 1:1:1:1 nmr quartet (attributable to ^{11}B splitting; dissolved diborane?) appearing at about 2.2 ppm is shifted too far downfield from the BH_4^- multiplet to be a spinning sideband and has a slightly larger coupling constant than the prominent BH_4^- absorptions. The origin
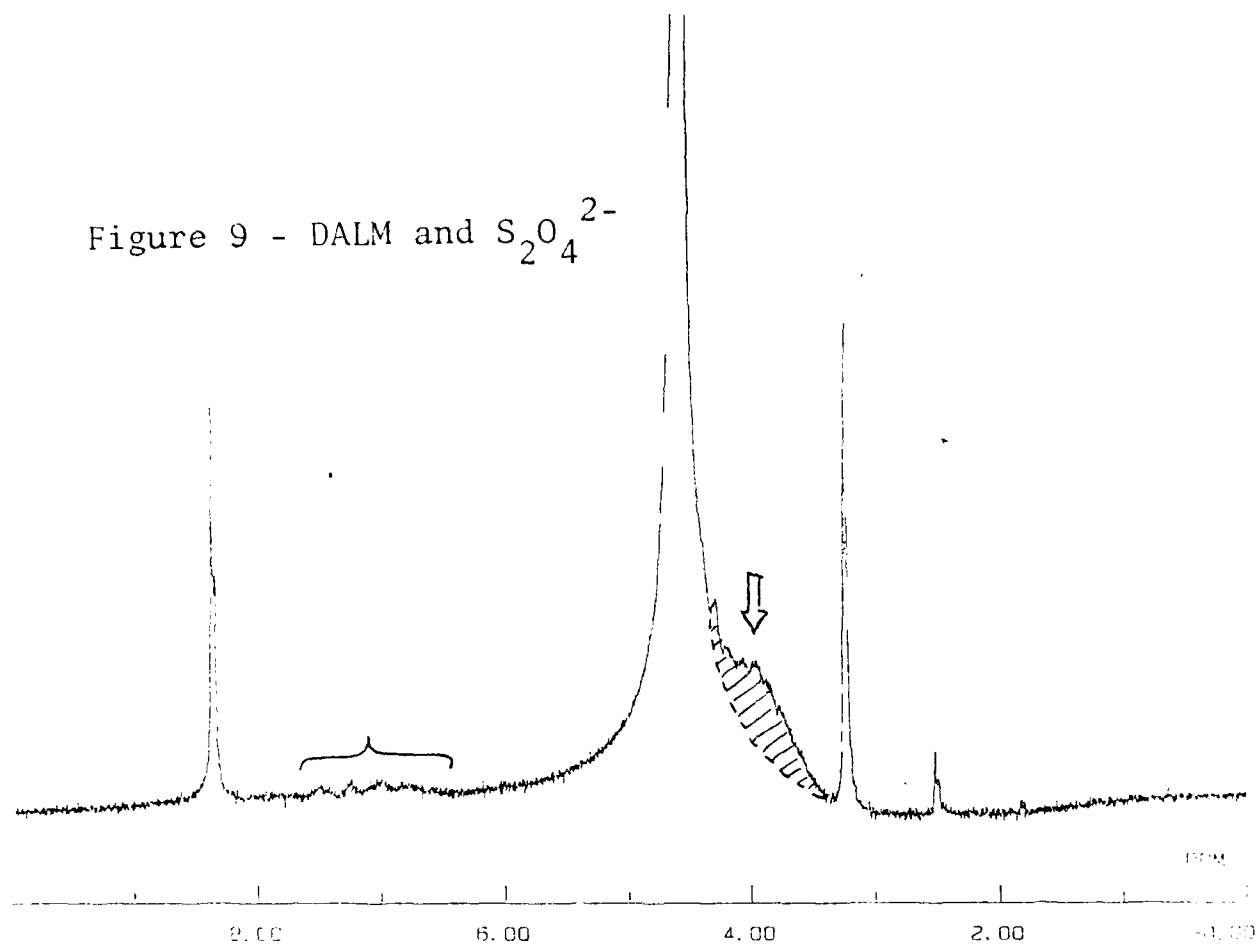
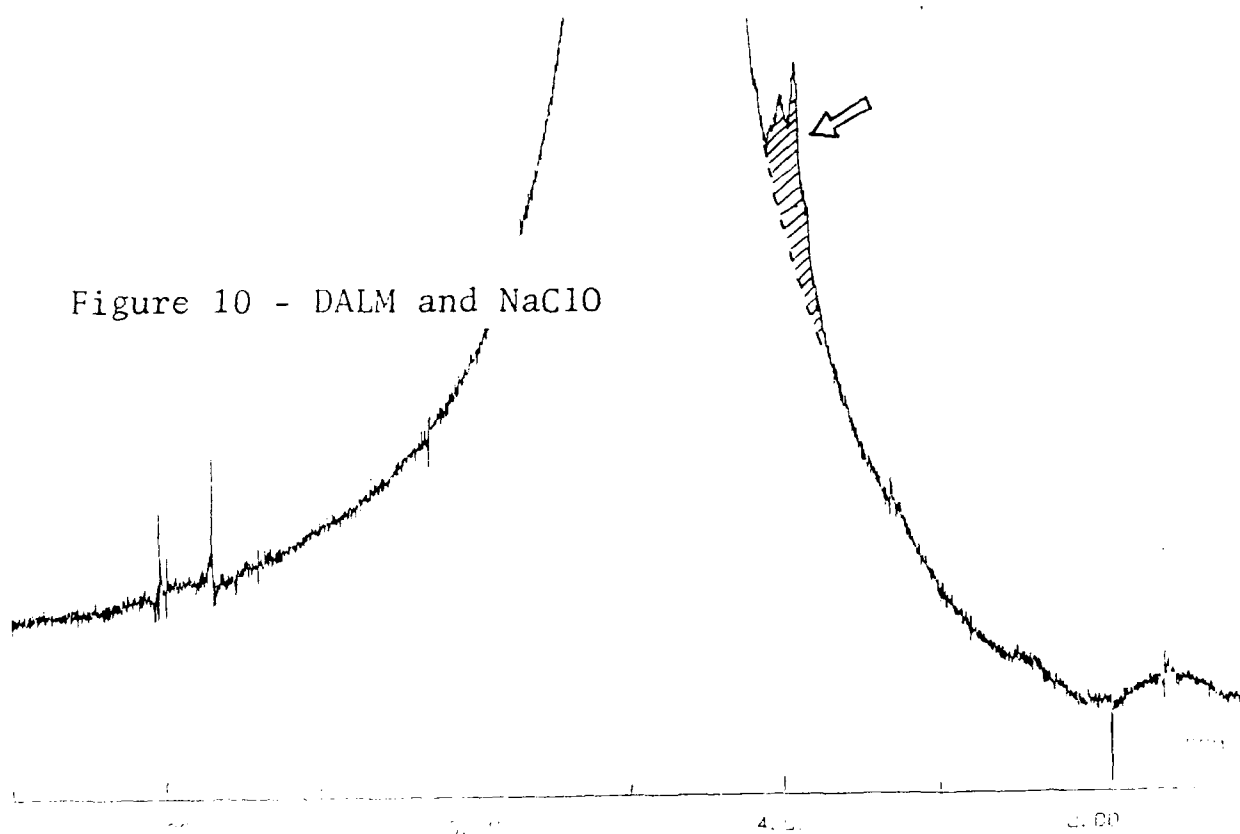Figure 9 - DALM and $S_2O_4^{2-}$
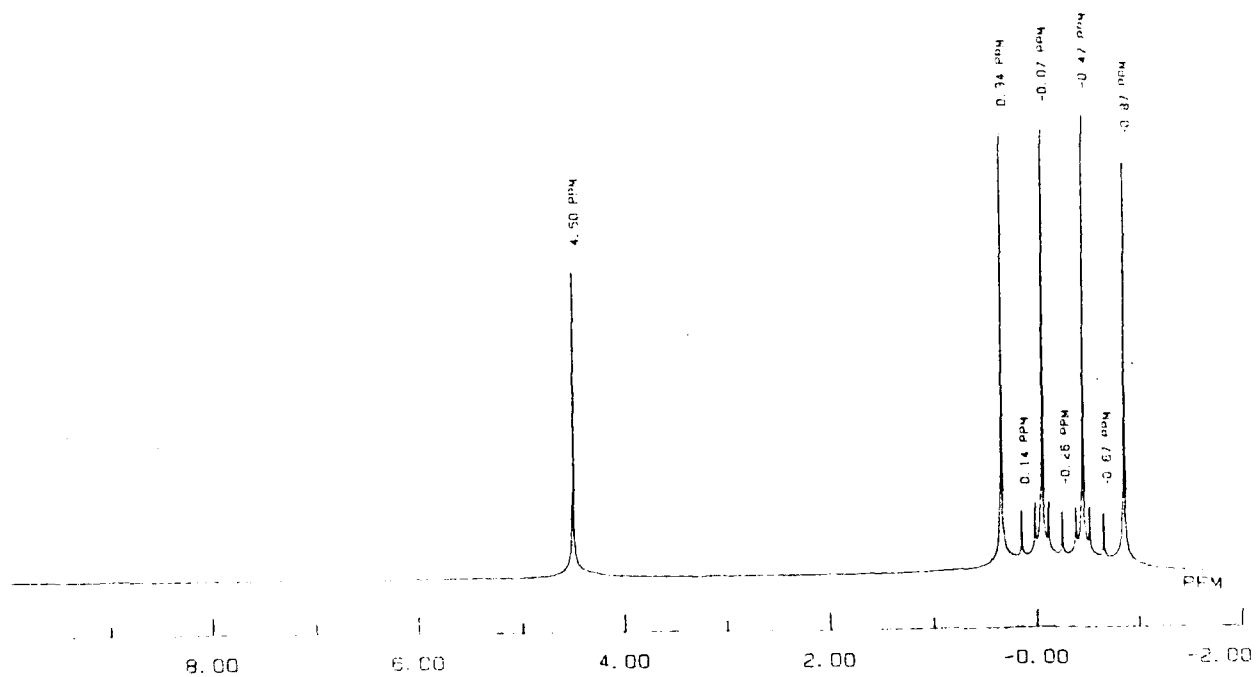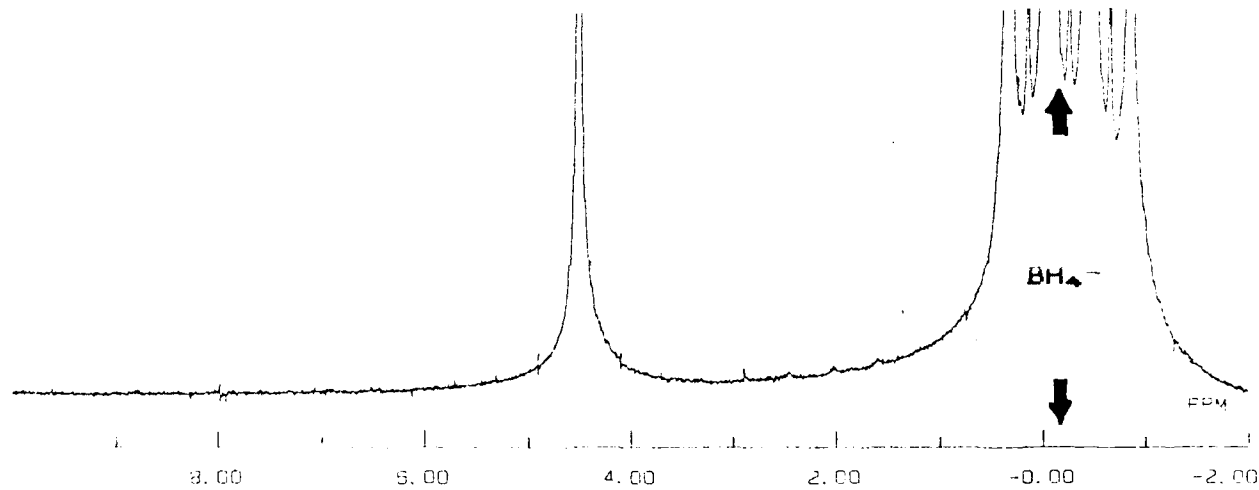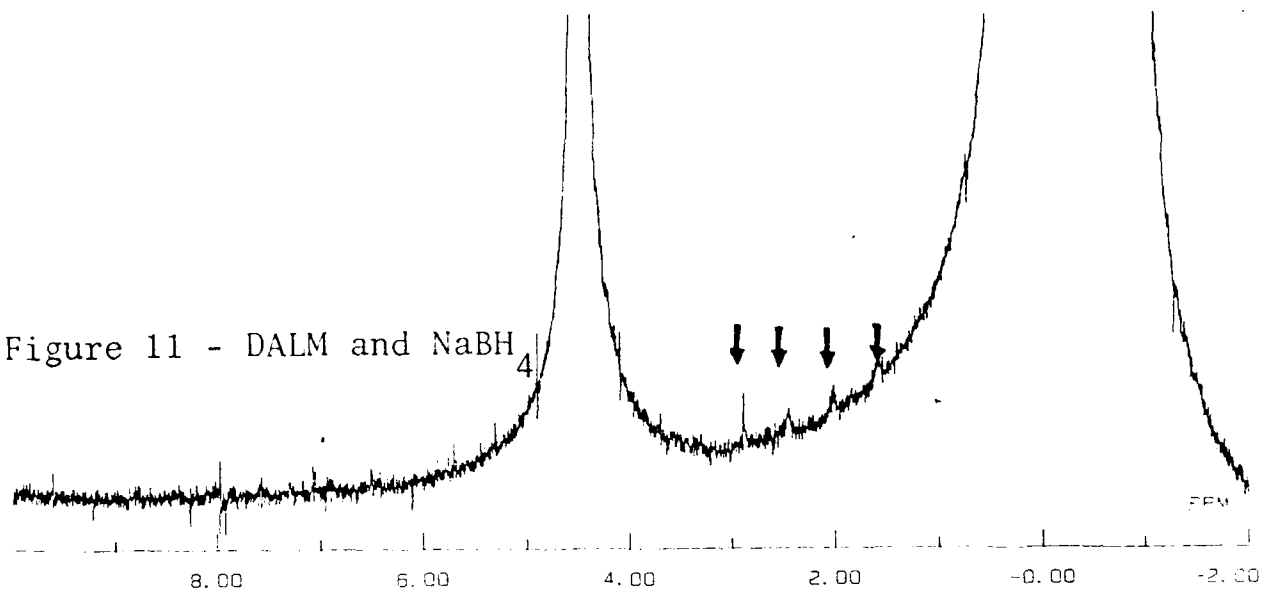
Figure 10 - DALM and NaClO

Figure 11 - DALM and NaBH$_4$

of this feature is unclear. The reagents $NaClO_3$ and $KBrO_3$ did not cause much bleaching, but for some unknown reason the nmr spectrum of DALM vanished in each case. All of these reagents were tried both at room temperature and in a boiling water bath, with the main differences appearing in the reaction rate.

4.5. Paramagnetism of the DALM-forming solid.

Proton nmr spin-lattice relaxation (T_1) measurements on aqueous solutions of DALM using the inversion/recovery pulse sequence obtained values closely comparable to that of pure water; thus, there is no evidence that the brown anion is a stable free radical, which should have caused the solvent T_1 to be much shortened. The strong esr absorption observed in the DALM-forming acetone precipitate (2) is almost certainly due to the slow thermal homolytic decomposition of the diazonium groups which are present in this mixture. Also, the esr spectrum is probably due to stable phenolic (phenoxyl) radicals (13), which should be produced by radical transfer, rather than the more unstable radicals formed directly in the diazonium decomposition process. The esr signal of DALM vanishes on dissolving the solid in water.

4.6. DALM and pulsed microwave fluences.

The most interesting feature of aqueous DALM solutions is the pulse of chemiluminescence in response to an applied pulse of microwave energy (3). This phenomenon occurs even if the pulse energy is too low to cause much heating on the sample, and it may be a result of the microwave acoustic effect (4), i.e., the

absorbed pulse generates an acoustic shock wave which in turn might initiate a sonochemical reaction. It has been demonstrated that alkaline aqueous solutions of luminol and hydrogen peroxide containing a small amount of a transition metal ion as the hydrated oxide are induced to chemiluminesce in response to acoustic irradiation. For example, a system based on Cu(II) was sensitive to heating effects, whereas the same system with Sc(III) in place of Cu(II) was differentially more sensitive to acoustic irradiations (14).

Luminol's chemiluminescence is known to be initiated by free radicals (5), and any process which generates radicals should activate luminol. Intense acoustic irradiations produce instantaneous local deviations of temperature amounting to thousands of degrees (4), and in view of the instability and energetic nature of the diazonium compounds (see 4.3.2. and Fig. 7) it would not be surprising if their solutions produced radicals in a strong acoustic field, especially at higher pH values.

During the reporting period an attempt was made to detect sonochemically-induced chemiluminescence from aqueous solutions of DALM and also from diazotized luminol, with mostly negative results. However, this question needs to be reconsidered since our arrangement for coupling light from the sonic irradiation compartment to the luminometer is not efficient. The metal ion systems cited above are about 1000-fold more luminescent than solutions of DALM or diazotized luminol, and while the instrument was more than adequate for characterizing the metal oxide systems, the diazotized entities are luminescing near the noise level. We

are currently outfitting the luminometer with a much more efficient flexible light guide for the purpose of characterizing DALM solutions. It is noted that aqueous solutions of diazotized luminol showed evidence of a slight acoustically induced luminescence at pH 5.5 and in the _absence_ of added H_2O_2. This needs to be re-examined with the more sensitive instrument.

The weak and extended chemiluminescence of the DALM polymer remains puzzling in view of clear evidence of the involvement of radicals during its formation reaction (2). It is hard to believe that the luminol moity could survive intact in such an environment, and DALM may contain mostly aminophthalic acid residues; in fact, the additional pendant carboxylate groups could explain the more anionic nature of this substance compared with the DAT polymer (see 4.2.). The properties of DALM might not be a result of the polymer alone but of the polymer interacting with other substances in these preparations.

5. RECOMMENDATIONS

Further research efforts should concentrate on attempts to fragment the DALM polymer. Also, the DALM-producing solid should be dissolved and subjected to nmr spectroscopy in a continuous variation of total reaction time to see if dimers, trimers and small oligomers are isolable. The foremost goal of any future work should be a structural characterization of the polymer. Some attention should also be given to the possibility that acoustic irradiations might homolytically decompose diazonium compounds, thus activating a chemiluminescent material such as luminol. It

should be worthwhile to characterize the product of a solid state polymerization of 3-diazonium-L-tyrosine and to see if this solid also produces an esr spectrum, as in the case of DALM. Finally, the effect of metal ions upon DALM's properties should be examined since these might enhance the observed sensitivity to pulsed microwave irradiations.

6. REFERENCES CITED

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APPENDIX A

Tabulation of net pi-charges for various 3-AT polymerization modes

STRUCTURE	......NET PI CHARGE*.....		
	C-2	C-5	C-6
3-AT	-.076	-.063	-.051
polyaryl amine	-.076	-.063	-.051
polyaryl ether	-.058	-.063	-.039
DOPA***	-.062	-.062	-.041
aryl polyester	-.026	+.001	-.043
diazo coupled	+.020	-.037	**
aryl diamine***	-.071	-.133	**
biphenyl dimer	-.003	-.048	-.008

*HMO computations of net charges. Only the carbons which have hydrogen substituents are shown here. The numbering of 3-AT's ring is retained in all of these structures (this table corresponds to the structures shown in Fig. 3). The polyaryl amine (Fig. 3A) should be very similar to 3-AT. The polyaryl ether (Fig. 3D) and DOPA might not show large chemical shift differences from the polyaryl amine (but both have inconsistent ionophoric properties). The other structures should show much modified aromatic chemical shifts.

**Does not have a hydrogen substituent.

***Monomers.

APPENDIX B

Estimation of the maximum absorption wavelengths for the p-bands of two benzenoid chromophores based on HMO Eigenvalues.

Compound	Band Gap*	p-Band Wavelength
benzene	2.000	206.8 nm
structure A phenol	0.942	439 nm**
structure A phenolate	0.778	532 nm**
structure B phenol	0.776	533 nm**
structure B phenolate	0.718	576 nm**

These values from zero-order computations are only crude approximations. See A. Streitwieser, Jr., "Molecular Orbital Theory for Organic Chemists", John Wiley and Sons, Inc., New York, 1967, p215. The transition from the highest occupied to the lowest unoccupied MO. The strong beta-band is near the violet/UV boundary, and (n-pi) transitions also occur. The phenol/phenolate titration at ca. pH 10 should cause a distinct red shift of the absorption bands.

**Calculated by proportion to the benzene p-band.
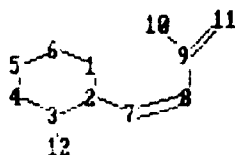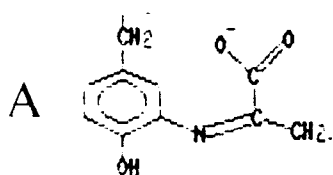
APPENDIX B continued

Total ground state pi energy: 6a + 8.000b
Total excited state pi energy: 6a + 6.000b
Total coulombic energy: 0.000b

Orbital energies
Level energy   No. Electrons

    1   2.000   2
    2   1.000   2
    3   1.000   2
    4   -1.000   0
    5   -1.000   0
    6   -2.000   0

APPENDIX B continued

A

(RING NUMBERING IS NOT SYSTEMATIC)

Total ground state pi energy: 14a + 26.209b
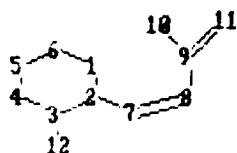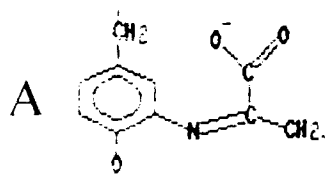Total excited state pi energy: 14a + 26.103b
Total coulombic energy: 4.500b

Orbital Energies
Level   Energy   No. Electrons

Level	Energy	No. Electrons
1	2.654	2
2	2.239	2
3	1.907	2
4	1.507	2
5	1.000	2
6	0.878	2
7	0.649	2
8	-0.293	0
9	-1.077	0
10	-1.183	0
11	-1.647	0
12	-2.133	0

APPENDIX B continued

A

Total ground state pi energy: 14a + 23.3646
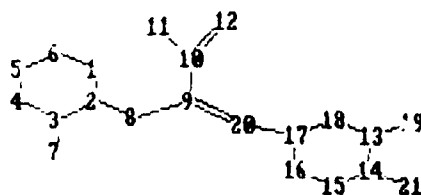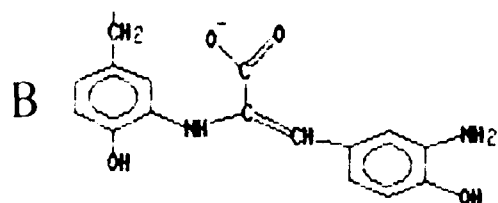Total excited state pi energy: 14a + 23.2696
Total coulombic energy: 3.5006

Orbital Energies
Level   Energy   No. Electrons

Level	Energy	No. Electrons
1	2.393	2
2	2.183	2
3	1.570	2
4	1.481	2
5	1.000	2
6	0.837	2
7	0.467	2
8	-0.311	0
9	-1.104	0
10	-1.199	0
11	-1.648	0
12	-2.170	0

APPENDIX B continued

B

Total ground state pi energy: 26a + 49.566b
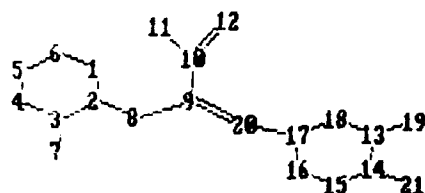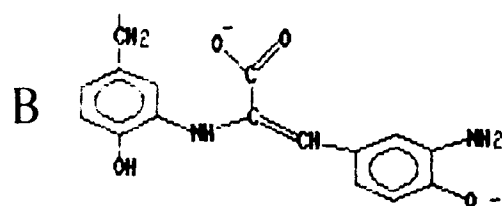Total excited state pi energy: 26a + 49.065b
Total coulombic energy: 9.000b

Orbital Energies
Level Energy   No. Electrons

Level	Energy	No. Electrons
1	2.788	2
2	2.687	2
3	2.485	2
4	2.138	2
5	2.049	2
6	1.820	2
7	1.680	2
8	1.104	2
9	1.000	2
10	0.901	2
11	0.755	2
12	0.591	2
13	0.284	2
14	-0.492	0
15	-1.066	0
16	-1.088	0
17	-1.149	0
18	-1.319	0
19	-1.820	0
20	-2.113	0
21	-2.234	0

APPENDIX B continued

Total ground state pi energy: 26a + 46.696b
Total excited state pi energy: 26a + 46.210b
Total coulombic energy: 8.000b

Orbital Energies
Level   Energy   No. Electrons

Level	Energy	No. Electrons
1	2.787	2
2	2.510	2
3	2.443	2
4	2.050	2
5	1.828	2
6	1.726	2
7	1.657	2
8	1.000	2
9	1.000	2
10	0.867	2
11	0.753	2
12	0.520	2
13	0.207	2
14	-0.511	0
15	-1.066	0
16	-1.092	0
17	-1.149	0
18	-1.344	0
19	-1.830	0
20	-2.115	0
21	-2.241	0